

REMARKS

Prior to this Preliminary Amendment, Claims 1-65 as presented in the annex to the International Preliminary Examination Report were pending. With this Preliminary Amendment, all pending Claims 1-65 are deleted and new Claims 1-69 are added. Payment for the fees associated with the addition of these claims has been directed on the Transmittal Letter to the United States Designated/Elected Office Concerning a Filing Under 35 U.S.C. §371 filed concurrently herewith.

Respectfully submitted,

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APPENDIX: VERSION WITH MARKINGS TO SHOW CHANGES MADE

[1. A method for introducing into a naturally non-isoflavonoid-producing plant species the enzyme catalyzing the aryl migration of a flavanone to form an isoflavanone intermediate or an isoflavone, comprising:

introducing a DNA segment encoding said enzyme into said plant to form a transgenic plant,
 5 wherein said transgenic plant expresses said DNA segment under the control of a suitable constitutive or inducible promoter when said transgenic plant is exposed to conditions which permit expression.]

[2. The method of Claim 1, wherein chalcone synthase, chalcone reductase, and chalcone isomerase genes are also expressed in said plant to cause in vivo formation of daidzein or a daidzein derivative.]

[3. The method of Claim 2, wherein said plant is further transformed to comprise said chalcone synthase, chalcone reductase, and chalcone isomerase genes.]

[4. The method of Claim 1 or 2, wherein said plant further comprises downstream genes to metabolize said formed isoflavanone intermediate or isoflavone to biologically active isoflavonoid derivatives or conjugates.]

[5. The method of Claim 4, wherein said downstream gene is selected from the group consisting of isoflavone *O*-methyltransferase, isoflavone 2'-hydroxylase, isoflavone reductase, and vestitone reductase.]

[6. The method of Claim 5, wherein said plant comprises downstream gene 4'-*O*-methyltransferase to form biochanin A or a biochanin A derivative.]

[7. A method for increasing the level of isoflavonoid compounds in naturally isoflavonoid-producing plants comprising:
 introducing a DNA segment encoding the enzyme catalyzing the aryl migration of a flavanone to yield an isoflavonoid to form a transgenic plant, wherein said transgenic plant expresses said
 5 DNA segment under the control of a suitable constitutive or inducible promoter when said transgenic plant is exposed to conditions which permit expression.]

[8. The method of Claim 7, wherein said isoflavonoid is selected from the group consisting of an isoflavonone intermediate, an isoflavone, an isoflavone derivative, and an isoflavone conjugate.]

[9. The method of Claim 1, 7 or 8, wherein said DNA segment comprises isolated genomic DNA.]

[10. The method of Claim 1, 7 or 8, wherein said DNA segment comprises recombinant cDNA.]

[11. The method of Claim 7-10, wherein said DNA segment comprises CYP93C gene.]

[12. The method of Claim 7-10, wherein said DNA segment is a *Medicago truncatula* homolog of a CYP93C gene.]

[13. The method of Claim 1-12, wherein said flavanone is liquiritigenin.]

[14. The method of Claim 1-12, wherein said flavanone is naringenin.]

[15. A method for synthesizing an isoflavanone intermediate or an isoflavone from a flavanone by expressing a recombinant CYP93C gene segment in a suitable bacterial, fungal, algal, or insect cell system.]

[16. A method of reducing the levels of isoflavonoid compounds in a naturally isoflavonoid-producing plant comprising introducing and expressing an antisense or gene silencing construct that contains an intact CYP93C gene or segments thereof into said plant.]

[17. The method of Claim 1, 11, 15 or 16, wherein said gene consists of the sequence from nucleotide 36 to nucleotide 1598 of the sequence depicted in SEQ ID NO:1.]

[18. The method of Claim 1, 12, 15 or 16, wherein said gene consists of the sequence from nucleotide 92 to nucleotide 1657 of the sequence depicted in SEQ ID NO:4.]

[19. A naturally non-isoflavonoid producing plant cell transformed by introducing a DNA segment encoding the enzyme catalyzing the aryl migration of a flavanone to form an isoflavanone intermediate or an isoflavone, wherein said transgenic plant cell expresses said DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.]

[20. The plant cell of Claim 19, wherein chalcone synthase, chalcone reductase, and chalcone isomerase genes are also expressed in said plant to cause in vivo formation of daidzein or a daidzein derivative.]

[21. The plant cell of Claim 20, wherein said plant cell is further transformed to comprise said chalcone synthase, chalcone reductase, and chalcone isomerase genes.]

[22. The plant cell of Claim 19-20, wherein said plant cell further comprises downstream genes to metabolize said formed isoflavanone intermediate or isoflavone to biologically active isoflavonoid derivatives or conjugates.]

[23. The plant cell of Claim 22, wherein said downstream gene is selected from the group consisting of isoflavone *O*-methyltransferase, isoflavone 2'-hydroxylase, isoflavone reductase, and vestitone reductase.]

[24. The plant cell of Claim 23, wherein said plant cell comprises downstream gene 4'-*O*-methyltransferase to form biochanin A or a biochanin A derivative.]

[25. A naturally isoflavonoid-producing plant cell transformed by introducing a DNA segment encoding the enzyme catalyzing the aryl migration of a flavanone to yield an isoflavonoid to form a transformed plant cell, wherein said transformed plant cell expresses said DNA segment under the control of a suitable constitutive or inducible promoter when exposed to conditions which permit expression.]

[26. The plant cell of Claim 25, wherein said isoflavonoid is selected from the group consisting of an isoflavanone intermediate, an isoflavone, an isoflavone derivative, and an isoflavone conjugate.]

[27. The plant cell of Claim 19, 25 or 26, wherein said DNA segment comprises isolated genomic DNA.]

[28. The plant cell of Claim 19, 25 or 26, wherein said DNA segment comprises recombinant cDNA.]

[29. The plant cell of Claim 19 or 25-28, wherein said DNA segment comprises CYP93C gene.]

[30. The plant cell of Claim 19 or 25-28, wherein said DNA segment is a *Medicago truncatula* homolog of a CYP93C gene.]

[31. A transgenic plant cell having reduced levels of isoflavonoid compounds, said plant cell transformed by introducing an antisense or gene silencing construct that contains an intact CYP93C gene or segments thereof into said plant cell.]

[32. The plant cell of Claim 29 or 31, wherein said gene consists of the sequence from nucleotide 36 to nucleotide 1598 of the sequence depicted in SEQ ID NO:1.]

[33. The plant cell of Claim 30 or 31, wherein said gene consists of the sequence from nucleotide 92 to nucleotide 1657 of the sequence depicted in SEQ ID NO:4.]

[34. An isolated gene or DNA segment comprising a portion which encodes a cytochrome P450 of the CYP93 family that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone, wherein said portion consists of nucleotide 36 to nucleotide 1598 of the sequence depicted in SEQ ID NO:1.]

[35. The gene or DNA segment of Claim 34, wherein said gene is the soybean gene encoding the enzyme catalyzing the aryl migration of liquiritigenin.]

[36. The gene or DNA segment of Claim 34, wherein said gene is the soybean gene encoding the enzyme catalyzing the aryl migration of naringenin.]

[37. A protein encoded by a portion of an isolated gene or DNA segment which encodes a cytochrome P450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone, wherein said portion consists of nucleotide 36 to nucleotide 1598 of the sequence depicted in SEQ ID NO:1.]

[38. An isolated gene or DNA segment comprising a portion which encodes a cytochrome P450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone, wherein said portion is a *Medicago truncatula* homolog of a CYP93C gene.]

[39. The gene or DNA segment of Claim 38 consisting of nucleotide 92 to nucleotide 1657 of the sequence depicted in SEQ ID NO:4.]

[40. The gene or DNA segment of Claims 38 or 39, wherein said gene is the *Medicago truncatula* gene encoding the enzyme catalyzing the aryl migration of liquiritigenin.]

[41. The gene or DNA segment of Claims 38 or 39, wherein said gene is the *Medicago truncatula* gene encoding the enzyme catalyzing the aryl migration of naringenin.]

[42. A protein encoded by a portion of an isolated gene or DNA segment which encodes a cytochrome P450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone, wherein said portion is a *Medicago truncatula* homolog of a CYP93C gene.]

[43. A transgenic plant cell transformed with an isolated gene or DNA segment which encodes a cytochrome P450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone, wherein said transgenic plant cell exhibits increased levels of an isoflavonoid when compared to the level of said isoflavonoid in plant cells of the same species which do not comprise said isolated gene or DNA segment.]

[44. A food comprising edible transgenic plant material capable of being ingested for its nutritional value, wherein said transgenic plant comprises plant cells according to claim 43.]

[45. A method of preparing a food comprising at least one isoflavonoid comprising: transforming a plant according to the method of claim 1 or 7, wherein said transgenic plant exhibits increased levels of an isoflavonoid when compared to the level of said isoflavonoid in plants of the same species which do not comprise said DNA segment, isolating said isoflavonoid and incorporating into said food.]

[46. A composition comprising at least a portion of a transgenic plant according to claim 43, wherein said composition is suitable for ingestion as a food stuff, a nutritional supplement, an animal feed supplement, or a nutraceutical.]

[47. A method of preparing a composition comprising an isoflavonoid suitable for administration as a food stuff, a nutritional supplement, an animal feed supplement, a nutraceutical, or a pharmaceutical, comprising: transforming a plant according to the method of claim 1 or 7, wherein said transgenic plant exhibits increased levels of an isoflavonoid when compared to the level of said isoflavonoid in

plants of the same species which do not comprise said DNA segment, isolating said isoflavonoid and incorporating into said compositions.]

[48. A method of using a transgenic plant according to claim 43 to provide a nutraceutical benefit to a human or animal administered said isoflavonoid.]

[49. The method of Claim 48, wherein said isoflavonoid is administered by ingestion of at least a portion of said plant.]

[50. The method of Claim 48, wherein said isoflavonoid is administered by ingestion of a composition comprising an isoflavonoid isolated from said plant.]

[51. A method for making a pharmaceutical preparation, comprising:
transforming a plant according to the method of claim 1 or 7, wherein said transgenic plant exhibits increased levels of an isoflavonoid when compared to the level of said isoflavonoid in plants of the same species which do not comprise said DNA segment, isolating said isoflavonoid and formulating said isoflavonoid to form a pharmaceutical preparation.]

[52. A method of transforming a plant with an isolated gene or DNA segment which encodes a cytochrome P450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone, wherein said transgenic plant exhibits increased levels of an isoflavonoid when compared to the level of said isoflavonoid in plants of the same species which do not comprise said isolated gene or DNA segment.]

[53. A method of Claim 52, wherein the nutritional value of said plant is increased.]

[54. A method of Claim 52, wherein the disease resistance in said plant is increased.]

[55. A method of Claim 52, wherein bacterial or fungal symbiosis in said plant is increased.]

[56. A method of claim 52, wherein said plant is a leguminous plant.]

[57. A method of claim 56, wherein the nodulation efficiency of said plant is increased.]

[58. A leguminous transgenic plant exhibiting increased nodulation efficiency, wherein said transgenic plant is transformed according to the method of Claim 52.]

[59. A transgenic plant of Claim 43 exhibiting an increased level of bacterial or fungal symbiosis.]

[60. A transgenic plant comprising at least one recombinant DNA sequence encoding a cytochrome P450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone, wherein said transgenic plant exhibits an increased level of an isoflavonoid when compared to the level of said isoflavonoid in plants of the same species which
5 do not comprise said recombinant DNA sequence.]

[61. Seed from a transgenic plant according to Claim 60.]

[62. Progeny from a transgenic plant according to Claim 60.]

[63. Progeny from seed of a transgenic plant according to Claim 60.]

[64. Use of a transgenic plant according to Claim 43 for the preparation of a nutraceutical preparation for achieving a nutritional effect.]

[65. Use of a transgenic plant according to Claim 43 for the preparation of a pharmaceutical preparation for achieving a therapeutic effect.]